Applicants respectfully disagree with this assessment. The H1 and H7 influenza hemagglutinin antigens used to protect mice and chickens, respectively, are representative of the antigens which can be used in the current invention, and were not intended to induce protection to other hemagglutinin subtypes. Appropriate antigens for other hemagglutinin subtypes, and for other viruses, pathogens, or infectious agents, can be used in the methods of the current invention.

Recent publications support the efficacy of the current methods in protection against viruses other than influenza. For example, Cox *et al.* demonstrated protection against a herpesvirus in cattle by injection with plasmid DNA encoding a bovine herpes virus-1 (BHV-1) glycoprotein (*J. Virology 67(9)*: 5664-5667 (1993); a copy of the publication is attached as Exhibit A). Inoculation provided protection, as shown by reduced clinical symptoms in all calves injected with the BHV-1 DNA.

Furthermore, the methods of the current invention are not limited to immunization of birds. Protection of both chickens (see particularly Examples 1-5 and 8) and mice (see particularly Examples 6 and 7) are described in the Specification. In addition, as described in the Declaration of Dr. Harriet Robinson, a co-inventor on the current application, further data shows protective immunization in ferrets. An unexecuted copy of the Declaration is attached as Exhibit B; the executed copy will be filed as soon as it is available.

As described in the Declaration, ferrets were immunized with a DNA transcription unit capable of expressing H1 hemagglutinin. Ferrets inoculated intramuscularly cleared virus more rapidly than control ferrets, indicating anti-influenza immunity. Ferrets inoculated with the gene gun were completely protected from challenge with influenza, as shown by an inability to

recover virus in nasal washes of inoculated animals post-challenge. Some of these ferrets showed that they were protected against infection even in the absence of neutralizing antibody in their pre-challenge sera.

The successful use of DNAs encoding influenza and herpesvirus proteins to raise protection against disease in birds and several different species of mammals, as described in the Specification, the attached paper (Exhibit A), and the Declaration of Dr. Robinson (Exhibit B), supports Applicants' position that the methods of the current invention can be used to protect any vertebrate animal.

Rejection of Claims under 35 U.S.C. 103

The Examiner rejected claims 1-4 as being unpatentable over King. The examiner indicated that:

> King describes a gene delivery technique in which the gene for gp120 protein of the AIDS virus was incorporated into a plasmid under the control of the CMV (cytomegalovirus) promoter sequence (page 5, abstract). Administration of the expression vector generated both cellular and humoral immune responses in mice to the gp120 protein. While King does not specifically describe a method of immunizing animals, it is stated that the technique is useful in immune therapy and may be an alternative to vaccination in cases of chronic infections and might also be applicable to disease states other than HIV. It would have been obvious to one of ordinary skill in the art, therefore, at the time the invention was made to employ the gene delivery technique in a method of immunizing animals against viral infection with the expectation, barring evidence to the contrary, that the technique would generate specific humoral and cellular immune responses to a variety of antigens.

Applicants respectfully disagree with this assessment.

The current invention pertains to a method of immunizing a vertebrate animal by administering a DNA

transcription unit that includes DNA encoding an antigen linked to a promoter region, whereby a humoral and/or cell-mediated immune response is elicited.

The King reference describes injection of a construct containing the gene for gp-120, a cytomegalovirus (CMV) early promoter sequence and tissue plasminogen activator (TPA) sequence, into the muscle of a mouse, and the resultant production of cytotoxic T cells in the mice against gp-120 protein. However, mice do not develop AIDS upon being infected with HIV; therefore, production of cytotoxic T cells against gp-120 in mice cannot be used to test for protective immunization. Production of cytotoxic T cells does not necessarily indicate that there will be protection upon challenge. Furthermore, King emphasizes that injection of the plasmid might be used "as a viable alternative to vaccination in cases of chronic viral infection", or that it might be used in immune therapy, i.e., for <u>treatment</u> in already-infected animals. These teachings are in contrast with the current invention, which pertains to protection against <u>infection</u>. King does not teach or describe any data demonstrating that inoculation with any gene for any particular epitope of any infectious agent would prevent infection, or provide antigenic protection against disease upon challenge.

<u>Rejection of Claims under 35 U.S.C 103</u>

The Examiner rejected Claims 15-18 as being unpatentable over WO 90/11092 in view of Huylebroeck et al., indicating that:

> given the importance of the influenza virus and the importance of the hemagglutinin in the generation of protective immune responses, it would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of WO 90/11092 on delivery of polynucleotides to vertebrate tissues, with the teachings of Huylebroeck et al. on the construction of non-retroviral

expression vectors encoding the influenza viral hemagglutinin, to include the DNA encoding viral hemagglutinin from influenza in a DNA transcription unit. A method of immunizing an animal including humans with the DNA transcription unit would have also been obvious, with the expectation, barring evidence to the contrary, that the DNA transcription unit would avoid the need to purify the HA antigen before use and the transcription unit would also generate humoral and cell-mediated immune responses when administered in vivo. To administer the transcription unit via the intranasal route would have been obvious given the fact that a natural route of infection for the influenza virus is through the nasal cavity. Combining preparations intended for vaccination purposes with physiologically acceptable carriers and excipients is well within the level of skill in the art.

Applicants respectfully disagree with this assessment. Obviousness is established only if the teachings of the cited art would suggest the claimed invention to one of ordinary skill in the art with a reasonable degree of certainty of successfully achieving the claimed results.

WO 90/11092 describes methods of delivering RNA or DNA polynucleotides into a vertebrate cell by interstitial delivery, exemplified by mRNA vaccination of mice to produce gp120 of HIV.

Huylebroeck et al. describe use of DNA in cell culture systems to produce the influenza virus HA, which is purified and inoculated into hosts. Huylebroeck also uses a recombinant, infectious, replication competent vaccinia virus vector to express HA, to produce the protein in a host and vaccinate the host.

The current invention pertains to a method of immunizing a vertebrate, such as a mammal, against an infectious agent, such as influenza virus, by administering a DNA transcription unit including an antigen, such as influenza virus hemagglutinin, linked to

a promoter; eliciting a humoral and/or cell-mediated immune response; and thereby protecting the vertebrate against disease.

One of ordinary skill in the art would not look beyond the general teachings of WO 90/11092 concerning delivery of polynucleotides, to the teachings of Huylebroeck et al. concerning influenza virus. There is no teaching or suggestion in WO 90/11092 that one of ordinary skill should look to influenza virus in particular, among all possible viruses and pathogens.

Furthermore, the methods of production of hemagglutinin described in Huylebroeck et al. differ in important aspects from the methods of generating desired antigens of the current invention: Huylebroeck et al. utilize cell culture systems to generate influenza hemagglutinin proteins used in vaccination; they do not teach or suggest that influenza hemagglutinin could be generated in an animal by DNA inoculstion, as in the current invention. Also, Huylebroeck et al. use an infectious agent, replication competent vaccinia virus, to express hemagglutinin in an animal. In contrast, the current invention uses DNA encoding only the particular antigens, such as hemagglutinin, to produce the protein in a vertebrate animal. This DNA does not encode replication-competent virus, and is not capable of replication in the host. One of ordinary skill in the art would not have been motivated by the teachings of Huylebroeck et al. to utilize solely the hemagglutinin DNA in order to generate antigen in the organism to be vaccinated.

Even if the references were improperly combined, one of ordinary skill in the art would not have a reasonable expectation of success. WO 90/11092 does not teach or describe successful protection against infection by immunization of any vertebrate through the introduction of

polynucleotides; one of ordinary skill in the art would not have had a reasonable expectation that utilization of DNA encoding a particular antigen, such as hemagglutinin, would result in protection of vertebrate animals against infection and disease. Applicants have, for the first time, shown that inoculation by administering a DNA tramscription unit encoding a desired antigen results in protection from disease caused by an infectious agent.

Conclusion

In view of the amendments and the arguments presented above, Applicants respectfully request that the Examiner reconsider and withdraw all rejections.

If the Examiner believes that a telephone conversation will expedite prosecution of this application, the Examiner is requested to call Applicants' Attorney at (617) 861-6240.

Respectfully submitted,

Patricia Granahan

Patricia Granahan
Attorney for Applicants
Registration No. 32,227
Telephone: (617) 861-6240

Lexington, Massachusetts 02173
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